

**New Paragraph for First Line of Specification**

**CROSS-REFERENCE TO RELATED APPLICATION**

A<sup>2</sup> The present application is a divisional application of Serial No. 08/981,340, filed 18 December 1997, which is a U.S. National Stage filing under 35 U.S.C. §371 of PCT/EP96/02605, which claims foreign priority to EP 952016566 filed on 19 June 1995.

**Clean Copy of Amended Specification - paragraph at page 4, line 32 - page 5, line 10**

08/981,340  
A<sup>2</sup> Although articular cartilage proteins are considered to be the target autoantigens competent of stimulating autoaggressive T cells involved in the destruction of articular cartilage in autoimmune diseases, it was not until the present invention that these MHC Class II binding T-cell epitopes associated with cartilage-responsive autoreactive T cells have been identified on the cartilage proteins, in particular on HAG and HCLP. The peptides according to the invention resemble these MHC Class II binding T-cell epitopes, thus providing T-cell reactive peptides which can be used in the peptide-induced T cell tolerance therapy. Accordingly, patients can be treated with the peptides according to the invention to induce specific T-cell tolerance not only to the administered peptides but to the target autoantigens HAG and HCLP as well. As other components of the immune system are not affected by the peptides according to the invention, the immune system of the patient will remain intact and will be able to protect the patient against other infections.

**Clean Copy of Amended Specification - paragraph at page 5, lines 15-32**

A<sup>4</sup> Peptides according to the invention have been described. Perin et al., FEBS Letters 206:73 (1986) describes the structural relationship between link proteins and proteoglycan monomers and discloses a peptide fragment obtained after tryptic digestion of the link protein. The peptide fragment has the amino acid sequence SSAGWLADRSVRYPIKARNXGG. Goetinck et al., J. Cell Biol. 105:2403-2408 (1987) discloses the peptides NAGWLSDGSVQYPITKPREP and DAGWLADGSVRYPISRPRKR which correspond to the amino acid residues Asn<sup>207</sup>-Pro<sup>226</sup> and Asp<sup>306</sup>-Arg<sup>325</sup> respectively of the primary structure of link protein. Said peptides were synthesized

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to study the interactions between link protein and hyaluronic acid and said amino acid residues were found to be involved in the binding of link protein to hyaluronic acid. Neame et al., J. Biol. Chem. 261(8):3519-3535, (1986) describes the elucidation of the primary structure of link protein from rat chondrosarcoma proteoglycan aggregate. Analysis of a triptic digest of the link protein revealed a fragment having the amino acid sequence GGLDWCNAGWLS DGSVQYPITKPR. Perides et al., J. Biol. Chem. Vol. 264, no. 10:5981-5987 (1989) describes the isolation and partial characterization of a glial hyaluronate-binding protein (GHBP). Tryptic digestion of GHBP results in several peptide fragments, one of which having the amino acid sequence EQLFAAYEDGF EQCDAGWLADQTVRYPIRAPRVGCY.

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**Clean Copy of Amended Specification - paragraph at page 6, lines 14-22**

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The peptides according to the invention can also be prepared by recombinant DNA techniques. A nucleic acid sequence coding for a peptide according to the invention or a multimer of said peptides is inserted into an expression vector. Suitable expression vectors are, amongst others, plasmids, cosmids, viruses and YAC's (Yeast Artificial Chromosomes) which comprise the necessary control regions for replication and expression. The expression vector can be brought to expression in a host cell. Suitable host cells are, for instance, bacteria, yeast cells, and mammalian cells. Such techniques are well known in the art, see for instance Sambrooke et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989.

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**Clean Copy of Amended Specification - paragraph at page 6, lines 24 to page 7, line 2**

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According to the invention, patients suffering from T-cell mediated destruction of the articular cartilage can be treated with a therapeutical composition [comprizing] comprising one or more peptides according to the invention and a pharmaceutical acceptable carrier. Administration of the pharmaceutical composition according to the invention will induce tolerance of the specific autoreactive T cells of these patients to the autoantigenic proteins in the articular cartilage under attack and other self antigens which display the identified MHC Class II binding T cell epitopes

characterized by one of the amino acid sequences of SEQ ID NO:1-6. More specifically, administration of the pharmaceutical composition according to the invention will induce tolerance of the specific autoaggressive T cells to the autoantigens HAG [end] and HCLP. The induced tolerance thus will lead to a reduction of the local inflammatory response in the articular cartilage under attack.

**Clean Copy of Amended Specification - paragraph at page 12, lines 1-14**

PBMC obtained from heparinized venous peripheral blood were isolated by standard centrifugation on a Ficoll-Paque gradient. Cells were cultured in three- or four-fold at a concentration of  $1,5 \times 10^5$  cells/well in DMEM/Ham's F12 medium supplemented with 10% heat-inactivated pooled human serum, L-glutamine, 2-ME and antibiotics in flatbottomed microtiter plates. Cells were incubated in medium alone or in the presence of PHA (2.5  $\mu\text{g/ml}$ ) or in the presence of antigens, including the chicken proteoglycan fraction, the chicken collagen fraction, sonicated *Mycobacterium tuberculosis* or the peptides HAG1, HAG2, HAG3 and HCLP1 in concentrations of 50  $\mu\text{g/ml}$ , 5  $\mu\text{g/ml}$  or 0.5  $\mu\text{g/ml}$ . Cultures were incubated in a total volume of 210  $\mu\text{l}$  for 4, 5, 6 or 7 days at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. Cultures were pulsed with 0.5  $\mu\text{Ci}$  ( $1.85 \times 10^4$  Bq) [<sup>3</sup>H]Thymidine ([<sup>3</sup>H]TdR) for the last 18 hours of cell culture. Cells were harvested on glassfibre filters and [<sup>3</sup>H]TdR incorporation was measured by gasscintillation. Note that counting by gasscintillation is fivefold less efficient compared to liquid scintillation. Therefore, filters were measured for 5 min (Packard Matrix 96  $\beta$ -counter, Meriden CT).